MODIFICATION OF HIV-1 PROTEASE INHIBITOR PHARMACOPHORE TO ASSESS ANTI-MALARIAL PROPERTIES

by
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A thesis submitted to the faculty of The University of Mississippi in partial fulfillment of the requirements of the Sally McDonnell Barksdale Honors College.

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Finally, I owe my deepest gratitude to my family and friends for their constant love and support through this experience. Thank you for your words of wisdom and your support when things were not going the way I had planned.

ABSTRACT

MARGARET ANN STREET: Modification of HIV-1 Protease Inhibitor Pharmacophore to Assess Anti-Malarial Properties
(Under the direction of John Rimoldi)

For years, the human immunodeficiency virus type 1 (HIV-1) and malarial parasites have both been prevalent in sub-Saharan Africa. Coinfections of HIV-1 and malaria are common in areas of geographic overlap as both infections seem to increase the severity of the other. HIV-1 treatments have shown to decrease the *Plasmodium* infections resulting in a lower malarial incidence. HIV-1 protease inhibitors target a crucial step in the life cycle required for viral maturation and have demonstrated a lower level of resistance compared with other therapies. Recent studies have shown the treatment of HIV-1 with protease inhibitors has resulted in inhibitory effects against the *Plasmodium falciparum* malaria parasite. With growing resistance to current anti-malarial drugs, the need for new compounds is crucial. In an effort to identify the key functional group in protease inhibitors responsible for malaria inhibition, the lopinavir analogs were designed around blocking the HIV-1 activity. The secondary alcohol required for HIV-1 activity was modified in each of the designed compounds in an effort to isolate the malarial inhibition. The compounds were submitted for biological evaluation in a malarial inhibition assay against the chloroquine-sensitive and chloroquine-resistant strains of *P. falciparum*. The modifications of the secondary alcohol within the protease inhibitor pharmacophore resulted in a decreased potency against the malaria protozoan,

demonstrating a need for further research on how the protease inhibitor targets *P. falciparum*.

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LIST OF ABBREVIATIONS

AIDS	acquired immunodeficiency syndrome
CD4+	cluster of differentiation 4 positive
D6	chloroquine sensitive strain of *P. falciparum*
ESI	electrospray ionization
HAART	highly active antiretroviral therapy
HIV-1	human immunodeficiency virus type-1
HPLC	high performance liquid chromatography
IC_{50}	half maximal inhibitory concentration
MS	mass spectrometry
NCNPR	National Center for Natural Products Research
NMR	Nuclear Magnetic Resonance
THF	tetrahydrofuran
TLC	thin-layer chromatography
RP-HPLC	reversed-phase high performance liquid chromatography
PI	protease inhibitor
pH	potential of hydrogen
PMA	phosphomolybdic acid
SI	selectivity index
T-cells	T-lymphocytes
UV	ultraviolet
W2	chloroquine resistance strain of *P. falciparum*
WHO	World Health Organization

INTRODUCTION

The human immunodeficiency virus type-1 (HIV-1) is a retrovirus that infects the cluster of differentiation 4 positive (CD4+) T-lymphocytes (T-cells) of the immune system. This impairs the immune system's ability to fight off infections leaving people living with HIV-1 vulnerable to other infections and diseases.[1] Infections associated with immunodeficiency are known as opportunistic infections as they take advantage of the weakened immune system. HIV-1 is a slowly progressing disease that continues to damage the immune system from the time of infection until the presence of a high viral load and opportunistic infections eventually lead to acquired immunodeficiency syndrome (AIDS).[2]

Many were skeptical regarding the high HIV-1 prevalence in African countries as the World Health Organization (WHO) prevalence maps for HIV-1 in the early 1990s showed very low levels in south Africa creating a lack of urgency. The 1990s soon became characterized by an HIV-1 epidemic with very high morbidity and over half of adult mortalities attributable to HIV-1.[3] At the end of 2017, more than thirty-six million people were living with HIV-1 and AIDS worldwide. The WHO African region, shown in Figure 1, remains the most severely affected with 1 in 25 adults living with HIV-1.[4]

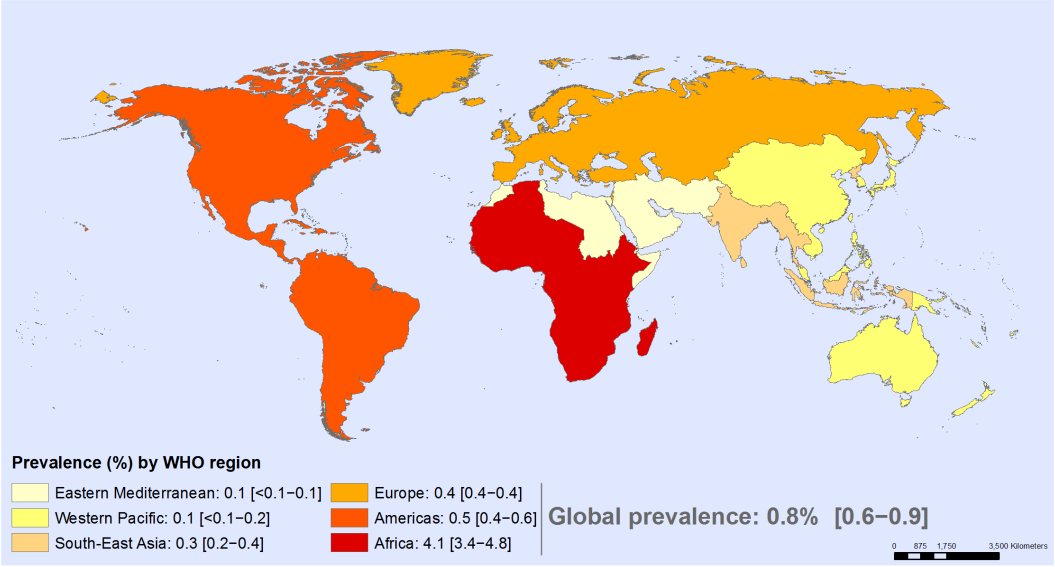


Figure 1: Prevalence of HIV-1 among adults ages 15-49 in 2017 by WHO region[4]

Development of HIV Protease Inhibitors

In the 1990s, few antiretrovirals were available so the primary management for HIV-1 was largely based on proper prophylaxis. Drug development focused on targeting each stage of the HIV-1 retroviral life cycle, shown in Figure 2. As more antiretrovirals were developed, the standard of care progressed from monotherapy to combination therapy.[5] The most current and most effective AIDS therapy is a form of combination therapy known as highly active antiretroviral therapy (HAART). The HAART combines the use of an HIV-1 protease inhibitor, reverse transcriptase inhibitor, and/or an integrase inhibitor. Protease inhibitors have proven to be one of the most important components in combination therapy due to the lower level of resistance compared with other therapies.[6]

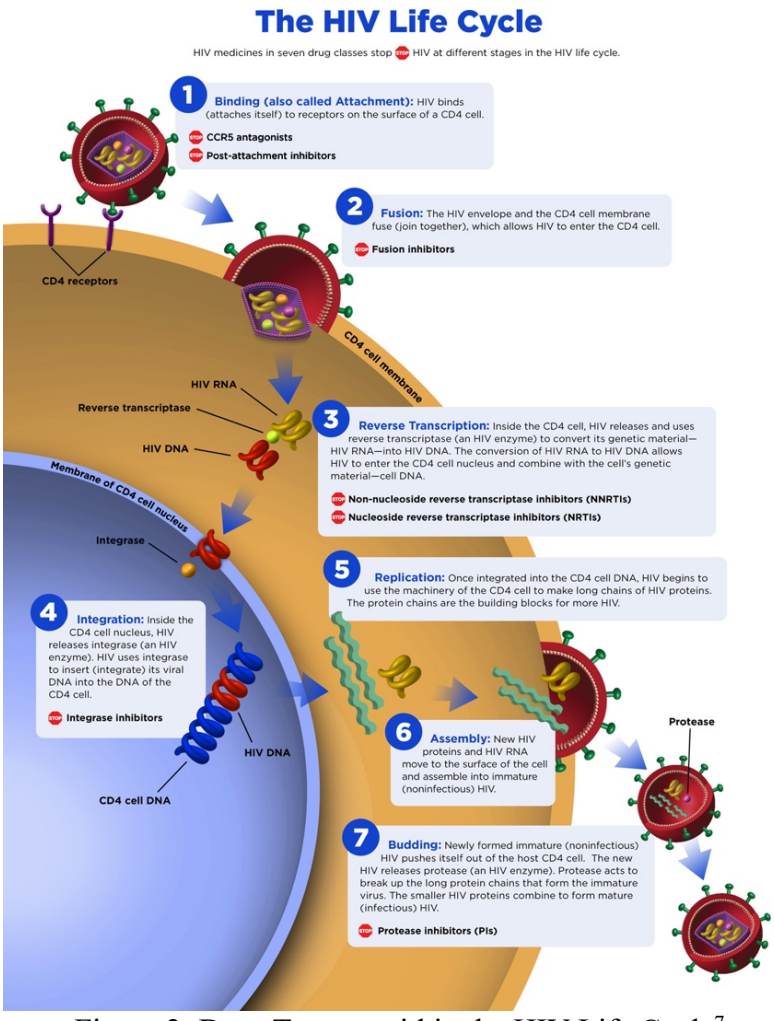


Figure 2: Drug Targets within the HIV Life Cycle[7]

HIV-1 proteases are essential in creating mature infectious viral particles. The HIV-1 protease is an aspartyl protease meaning it contains an aspartic acid in the active site. The protease is assembled in a symmetric homodimer with two identical subunits of ninety-nine amino acids.[8] The protease peptides have a superimposable secondary structure that creates a pocket designed to fit the substrate binding regions. This pocket allows the protease to recognize the skewed shape of the peptide rather than targeting a specific amino acid sequence. The protease active site is covered by two flexible ß-flaps that must be opened to allow the substrate to enter. The polyprotein precursor is cleaved

and digested into protease, reverse transcriptase, and integrase. The protease inhibitor complexes with the HIV-1 protease, shown in Figure 3, to block the active site preventing viral maturation. The secondary hydroxyl group of the inhibitor interacts with the protease via hydrogen bonding with the carboxyl group of the active site aspartic residues.[6]

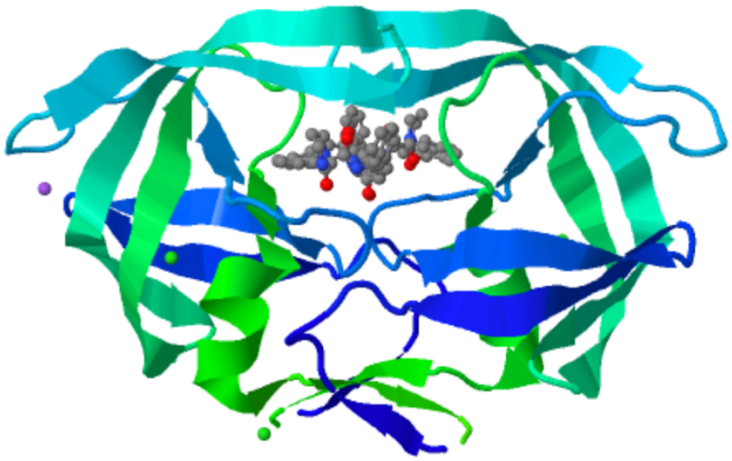


Figure 3: HIV-1 protease structure in complex with lopinavir
From PDB 6DJ1[9]

The pharmacophore of a molecule is the spatial arrangement critical for interactions in a specific binding site for a target receptor. Pharmacophores are often considered the largest common feature shared among a set of active molecules.[10] The majority of FDA approved HIV-1 protease inhibitors contain the hydroxyethylamine pharmacophore, highlighted in blue in Figure 4, designed to mimic the substrate transition state in order to interact with the protease active site. The secondary hydroxyl group of the pharmacophore is crucial for its interaction with the carboxyl group of the HIV-1

protease active site.[6] If the hydroxyethylamine pharmacophore is removed or substantially altered, the resulting ligands will not interact with the binding site of the target receptor therefore eliminating the inhibitory effects.

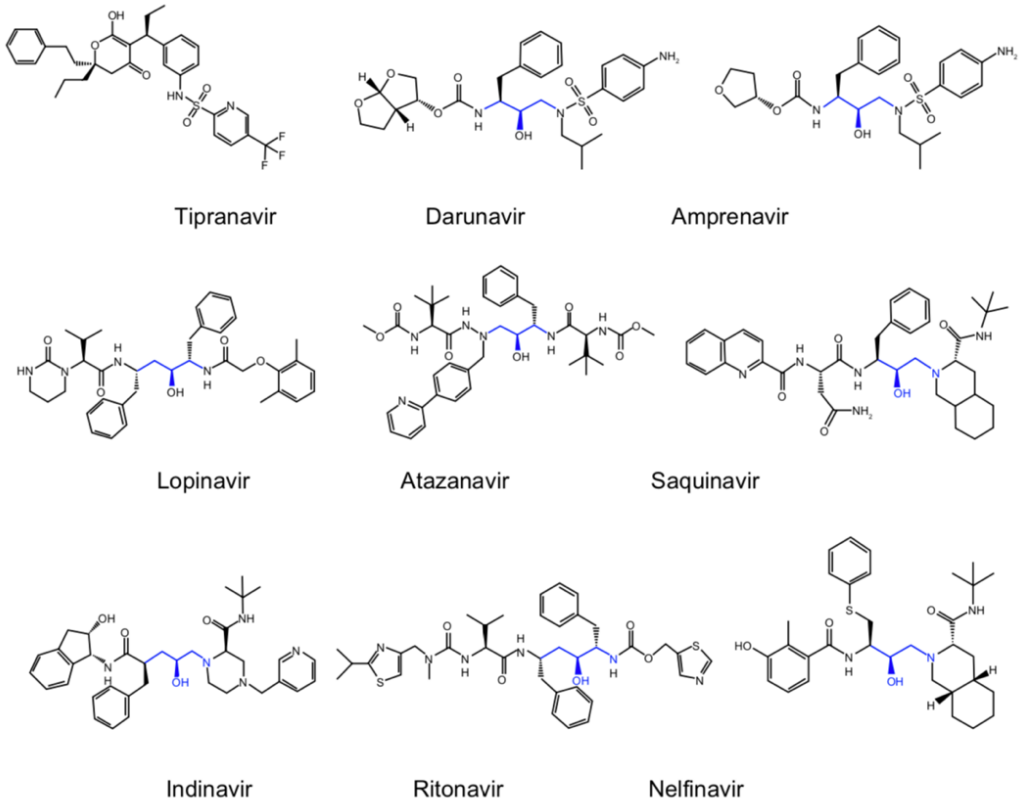


Figure 4: Nine FDA approved HIV-1 protease inhibitors[11]

Interaction of HIV-1 and Malaria

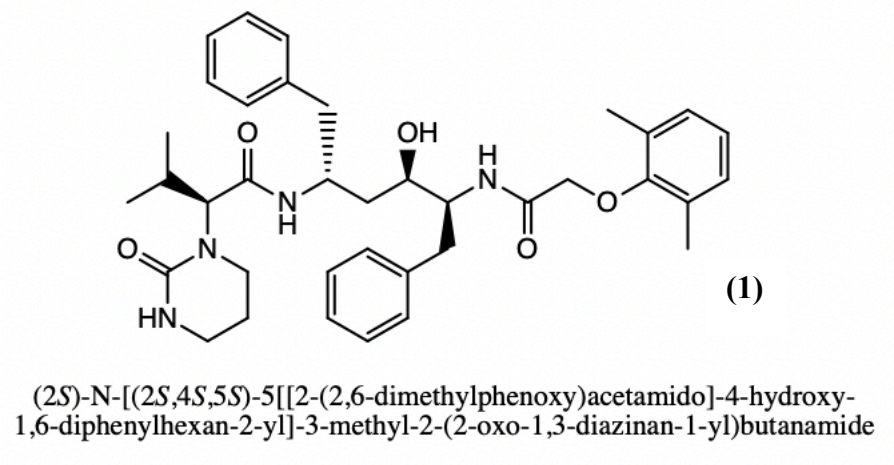
HIV-1 and malaria have proven to be two of the deadliest diseases, both of which are prevalent in sub-Saharan Africa. According to statistics from 2013, sub-Saharan Africa contains only 12% of the global population yet is burdened with 71% of the global

HIV-1 infection. Although HIV-1 infection trends in sub-Saharan are declining, almost 80% of those living with HIV-1 worldwide are located in southern and eastern Africa.[12]

Co-infections with HIV-1 and malaria have become common in sub-Saharan Africa with each enhancing the other's severity. The geographic overlap between HIV-1 and *Plasmodium* species has contributed to the spread of both diseases and led to an increase in coinfections.[13] HIV-1 infected individuals are more susceptible to malaria infections due to immune deficiency, which should lead to an increase in the frequency of coinfections with malaria.[14] Malaria infections create an ideal environment for HIV-1 replication, accelerating the progression of HIV-1. Malaria infections increase CD4+ T-cell activation to induce a strong inflammatory immune response. With this synergistic effect enhancing the harm to the patient, development of treatment options for co-endemic settings are crucial and the effectiveness of antiretroviral drugs in malarial settings needs to be tested.[15]

Current treatment options for malaria control are precarious, so recent measures have been taken to develop new or to repurpose existing drugs for valuable interventions in controlling the spread of infections. All recommended drug combinations for HIV-1 treatment have been shown to significantly decrease *Plasmodium* infections with protease inhibitors resulting in the lowest clinical malaria incidence.[13] Protease inhibitors are strong antiretrovirals with effective HIV-1 treatment but have also demonstrated effective *in vitro* activity against some malarial strains of *Plasmodium falciparum*.[15] According to a study performed in 2012, the lopinavir-ritonavir combination drug decreased the risk for malaria infections by roughly forty percent.[16] The protease inhibitor, lopinavir, is prepared as a combination formula with ritonavir. Ritonavir is a strong enzyme inhibitor

that increases with bioavailability of lopinavir, making lopinavir more effective against viral infections.[17] Of the protease inhibitors tested, lopinavir, shown in Figure 5, was the most potent antimalarial inhibitor, demonstrating a half maximal inhibitory concentration (IC_{50}) nearly tenfold below the trough blood concentration achieved with standard dosing.[18] The inhibitory concentration results indicated lopinavir was one of the most promising for antimalarial development, which was the reason lopinavir was chosen for this project.



(2*S*)-N-[(2*S*,4*S*,5*S*)-5[[2-(2,6-dimethylphenoxy)acetamido]-4-hydroxy-1,6-diphenylhexan-2-yl]-3-methyl-2-(2-oxo-1,3-diazinan-1-yl)butanamide

Figure 5: Structure and IUPAC name of Lopinavir

MATERIALS AND METHODS

General Methods

Each reaction was monitored for consumption of starting material or product formation using thin-layer chromatography (TLC) techniques and detection with ultraviolet (UV) light at 254 nM (lopinavir is UV active due to the presence of aromatic rings). The TLC plates were stained with phosphomolybdic acid (PMA) or potassium permanganate and heated to aid visualization of the compound.

All commercially available starting materials and reagents were purchased at the highest commercial quality and used without further purification unless otherwise noted. With many of the reagents being sensitive to moisture, all of the reactions were performed under standard anhydrous conditions. Standard anhydrous conditions indicate the reactions were performed in flame-dried glassware evacuated and cleared with an inert blanket of argon while using commercially available anhydrous solvents. Three analogs, shown in Figure 6, were synthesized from lopinavir using procedures derived from immunochemically equivalent compounds.[19] Lopinavir was deprotonated using sodium hydride or trichloroacetyl isocyanate and the appropriate alkylating agent was added to synthesize the desired analog.

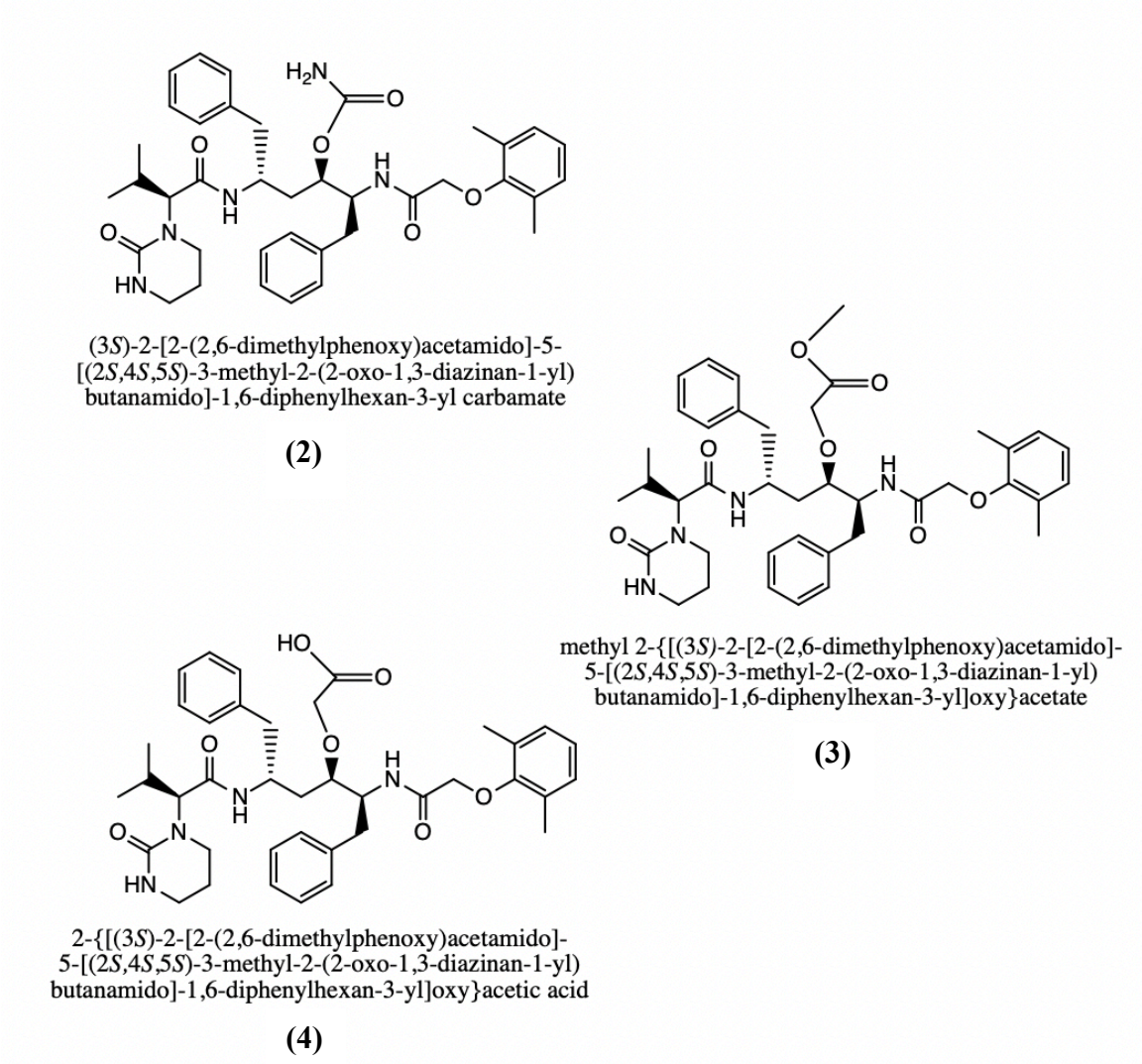


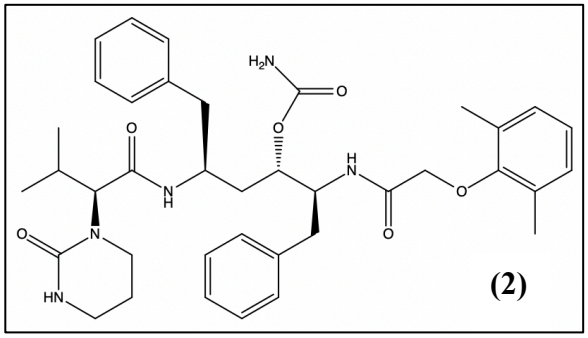
Figure 6: Synthesized Analogs of Lopinavir

Molecular weight analysis was performed using a Waters Micromass Quattro Micro mass spectrometer with either positive (ESI+) or negative (ESI-) electrospray ionization. A Bruker 400 MHz Avance Nuclear Magnetic Resonance (NMR) spectrometer was used in acquiring the proton (^{1}H) and carbon (^{13}C) NMR spectral data, which was then processed using MNova (MestReNova) software. A Teledyne ISCO CombiFlash Rf+ PurIon was used to conduct preparative high performance liquid

chromatography (HPLC) in separating and purifying the desired compounds from the product mixture. The desired compounds were assessed for inhibition against the *Plasmodium falciparum* protozoan using the antimalarial assay conducted by the National Center for Natural Product Research (NCNPR) at the University of Mississippi.

Experimental Methods

Lopinavir Carbamate

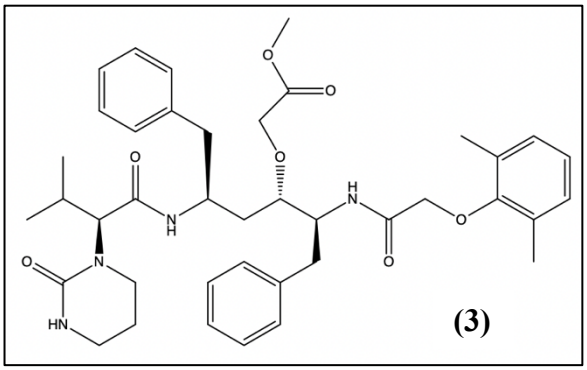


(2)

Under standard anhydrous conditions, commercially available lopinavir (100 mg, 0.159 mmol) was dissolved in dichloromethane (1 mL) and cooled in an ice bath to 0 ºC. Trichloroacetyl isocyanate (40 µL, 0.318 mmol) was slowly added to the solution. The reaction mixture was allowed to reach room temperature as it stirred for four hours. The crude product was concentrated under reduced pressure to produce lopinavir-o-trichloroacetylcarbamate. Potassium carbonate (455 mg, 3.3 mmol), methanol (4.5 mL), and water (1.5 mL) were added to the crude product at 0 ºC. The reaction mixture was allowed to stir at 0 ºC for one hour and at room temperature for eighteen hours. The mixture was concentrated under reduced pressure. Chloroform (100 mL) and water (30 mL) were added to the residue. The mixture was transferred to a separatory funnel and extracted 3 times with 20 mL of chloroform. The organic layers were combined and dried over magnesium sulfate. The organic layers were concentrated in a vacuum to give 115 mg of crude product. Purification of 50 mg of the crude product was accomplished using RP-HPLC with a

mobile phase consisting of a water-acetonitrile gradient. The mass of each fraction was analyzed, and the product fraction was lyophilized to remove water. From the 50 mg of crude material, 24 mg of the desired product was obtained (48% yield). **MS (ESI+)** *m/z* = 694.55 $[M+Na]^+$ **^{1}H NMR** (500 MHz, Methanol-*d*4) δ 7.81 (d, *J* = 9.2 Hz, 1H), 7.36 – 7.19 (m, 9H), 7.21 – 7.13 (m, 1H), 7.01 (d, *J* = 7.4 Hz, 2H), 6.98 – 6.91 (m, 1H), 5.02 (ddd, *J* = 8.1, 6.2, 2.3 Hz, 1H), 4.84 (s, 1H), 4.45 (dt, *J* = 10.1, 5.2 Hz, 1H), 4.33 (d, *J* = 11.2 Hz, 1H), 4.15 (d, *J* = 14.9 Hz, 1H), 4.07 (d, *J* = 14.9 Hz, 1H), 3.21 – 3.10 (m, 2H), 3.05 (ddd, *J* = 11.5, 7.0, 4.0 Hz, 1H), 2.99 – 2.77 (m, 4H), 2.59 (dd, *J* = 13.6, 9.8 Hz, 1H), 2.18 (s, 6H), 2.17 (d, *J* = 10.1 Hz, 1H), 1.89 – 1.75 (m, 2H), 1.73 – 1.64 (m, 1H), 1.50 (dt, *J* = 11.2, 6.3 Hz, 1H), 0.91 (d, *J* = 6.5 Hz, 3H), 0.84 (d, *J* = 6.7 Hz, 3H). **^{13}C NMR** (126 MHz, MeOD) δ 170.38, 170.29, 169.61, 157.73, 157.11, 154.35, 138.50, 137.82, 130.32, 129.21, 129.05, 128.70, 128.01, 127.93, 126.16, 125.73, 124.44, 72.99, 69.55, 62.59, 51.70, 51.62, 48.16, 47.99, 47.82, 47.65, 47.48, 47.31, 47.14, 40.48, 40.17, 39.40, 37.84, 37.49, 25.53, 21.17, 18.82, 17.60, 15.05.

Lopinavir Methyl Acetate

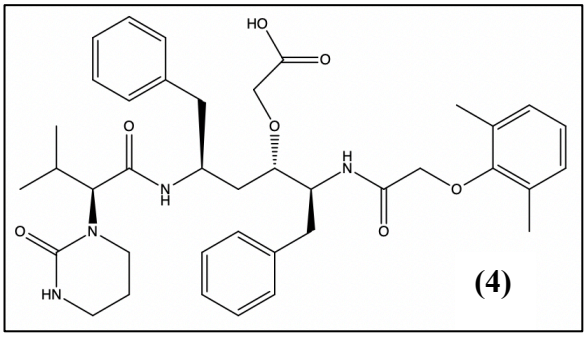


(3)

Under standard anhydrous conditions, commercially available lopinavir (80 mg, 0.127 mmol) was dissolved in THF at room temperature. Sodium hydride, 60% in oil, (24 mg) was added and the mixture stirred for one hour at room temperature. Methyl bromoacetate was added and the reaction stirred at room temperature for twenty-four hours. The mixture was quenched

with a 50mM phosphate buffer (sodium and potassium) and stirred at room temperature for one hour. The reaction mixture was transferred to a separatory funnel and extracted 4 times with 10 mL of ethyl acetate. The organic layers were combined and dried with magnesium sulfate. The organic layers were concentrated in a vacuum to give 100 mg of crude product. The product was then purified using preparative TLC using 5% isopropyl alcohol in chloroform. **MS (ESI+)** *m/z* = 724.56 $[M+Na]^+$.**^{1}H NMR** (400 MHz, Methanol-d4) δ 7.31 (dd, J = 17.5, 7.2 Hz, 5H), 7.25 (d, J = 4.4 Hz, 5H), 7.23 – 7.16 (m, 1H), 7.05 – 6.90 (m, 5H), 4.45 (s, 2H), 4.29 (dd, J = 28.5, 13.7 Hz, 3H), 4.19 – 4.11 (m, 2H), 4.07 (d, J = 14.8 Hz, 1H), 3.78 (s, 3H), 3.66 (s, 2H), 3.55 (d, J = 9.5 Hz, 1H), 3.17 (s, 2H), 3.15 – 3.01 (m, 1H), 3.04 (s, 1H), 2.97 (dd, J = 13.9, 4.9 Hz, 1H), 2.87 – 2.79 (m, 3H), 2.70 – 2.59 (m, 1H), 2.37 – 2.25 (m, 1H), 2.19 (d, J = 11.9 Hz, 5H), 2.19 (s, 6H), 1.92 (s, 0H), 1.84 (t, J = 12.6 Hz, 1H), 1.72 (d, J = 11.3 Hz, 1H), 1.55 (s, 15H), 1.32 (d, J = 6.8 Hz, 7H), 0.96 – 0.88 (m, 1H), 0.85 (dd, J = 6.7, 2.5 Hz, 7H).

Lopinavir Methyl Acetic Acid



(4)

Under standard anhydrous conditions, commercially available lopinavir (80 mg, 0.127 mmol) was dissolved in tetrahydrofuran (3 mL) at room temperature. Sodium hydride, 60% in oil, (19 mg) was added to the solution. The reaction stirred for one hour at room temperature. Methyl bromoacetate was added and the reaction mixture continued to stir at room temperature for ninety-six hours. Potassium phosphate was added followed by lithium hydroxide monohydrate and the reaction mixture stirred at room temperature for an

additional ninety-six hours. The reaction mixture was concentrated under pressure. Approximately six drops of 20% phosphoric acid were added to acidify the aqueous residue to a pH of 5-6 and extracted 5 times with 15 mL of chloroform. The organic layers were combined and dried over magnesium sulfate and concentrated in a vacuum to give 107 mg of crude product. The crude product was purified using preparative TLC using isopropyl alcohol, trifluoroacetic acid, and chloroform. **MS (ESI-)** *m/z* = 685.42 $[M-H]^-$

Anti-Malarial Bioassay

The antimalarial screening was completed by the National Center for Natural Products at the University of Mississippi. The NCNPR antimalarial screening was funded by United States Department of Agriculture, Agricultural Research Service Specific Cooperative Agreement, Grant Number 58-6408-1-603. The antimalarial screening tests a sample's ability to inhibit the chloroquine sensitive or chloroquine resistant *Plasmodium falciparum* protozoan. The primary screen tests crude extracts at 15,867 ng/mL against the D6 *P. falciparum* strain. The percent inhibitions are calculated relative to negative and positive controls. If the extract shows greater than fifty percent inhibition, it proceeds to the secondary assay.

In the secondary assay, the pure column fractions were dissolved to a concentration of 2 mg/mL. The samples were tested to determine the half maximal inhibitory concentration (IC_{50}) for both the chloroquine-sensitive (D6) and the chloroquine-resistant (W2) clones of *P. falciparum*. Analogues were evaluated for *in vitro* cytotoxicity against mammalian kidney cell line (VERO) up to a concentration of

4760 ng/mL via a neutral red assay. The IC_{50} is reported as the test concentration in nanograms per milliliter that provides a fifty percent inhibition of the protozoan relative to negative and positive controls. The positive controls in this assay were the antimalarial drug controls chloroquine and artemisinin. The selectivity index (SI) is calculated as a ratio of VERO IC_{50} to the IC_{50} of either D6 or W2 *P. falciparum* strain. The VERO IC_{50} is the concentration at which fifty percent of the viable cells die due to cytotoxicity. A high SI is favorable as the compound will have a higher selectivity towards the pathogen compared to the host.

RESULTS AND DISCUSSION

Three target analogues were successfully synthesized from lopinavir, which include lopinavir carbamate, lopinavir methyl acetate, and lopinavir methyl acetic acid. For each reaction, the secondary alcohol of lopinavir was targeted for deprotonation in order to add the desired group to the product molecule. Lopinavir (1) was deprotonated using sodium hydride or trichloroacetyl isocyanate and the appropriate alkylating agent was added to form the carbamate (2), methyl acetate (3) and methyl acetic acid (4) analogs. The structures of the final products were confirmed using MS and NMR spectrometry.

The hydroxylethylamine pharmacophore was modified to block the antiretroviral activity in an attempt to isolate the antimalarial activity for amplification. If the analogs were submitted for HIV-1 assays, the elimination of the hydroxylethylamine pharmacophore would likely result in the analogs being devoid of HIV-1 inhibitory activity. With the antiretroviral pharmacophore inhibited, the compounds were subjected to a biological assay to determine the concentration needed to acquire antimalarial activity. The results from the biological evaluation screening, shown in Table 1, indicate the modification of the hydroxyl group decreases the inhibitory effects against the malaria *Plasmodium falciparum*. This proves the secondary hydroxyl group is not only necessary for the antiretroviral activity but also for the antimalarial activity. The unmodified lopinavir demonstrated the greatest potency against both the D6 and W2

strains at concentrations of 4.26 μM and 2.1 μM, respectively. Previous *in vitro* studies have the IC_{50} of lopinavir against the W2 strain as being 2.7 μM, which is relatively high compared with its HIV activity of 0.005 μM.[20] The modification with the methyl acetate and methyl acetic acid demonstrated a decrease in potency for the *P. falciparum* D6 and W2 strains as the concentration needed for inhibition exceeded the limitations of the assay. Although the IC_{50} increased for the methyl acetate and methyl acetic acid, the analogs likely still have sufficient antimalarial activity compared to other protease inhibitors. Lopinavir has a relatively high activity against malaria compared with other HIV-1 protease inhibitors, which average at 17μM.[20]

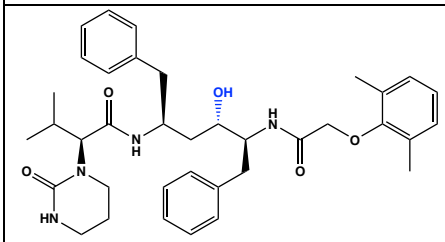
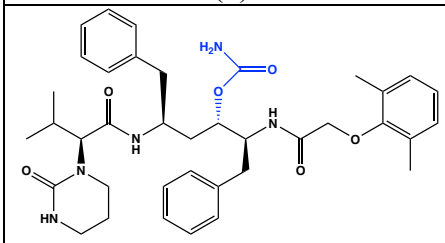
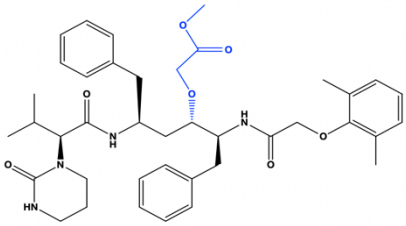
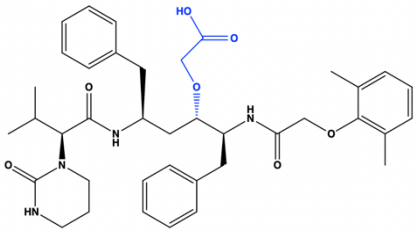
Compound	*P. falciparum* D6 IC_{50}	*P. falciparum* D6 SI	*P. falciparum* W2 IC_{50}	*P. falciparum* W2 SI
 (1)	2681	>1.8	1324.3	>3.6
 (2)	>4760	1	3563.1	>1.3
 (3)	>4760	1	>4760	1
 (4)	>4760	1	>4760	1

Table 1. Antimalarial screening results from NCNPR. All concentrations are in nanograms per milliliter.

The high SIs calculated for lopinavir at 1.8 and 3.8 demonstrate an achievable inhibitory concentration with minimal cytotoxicity effects. Although the screening was limited by the concentration range, the values indicate lopinavir methyl acetate and

lopinavir methyl acetic acid would require at least a four-fold increase in the drug concentration compared with lopinavir.

All three of the synthesized analogs are increased in size compared with lopinavir due to the addition of the larger functional groups. This increase in size could be interfering with the interaction of the inhibitor and malaria, causing a decrease in inhibitory activity. The change in the hydroxyl group decreases hydrogen bonding which likely interferes with the inhibitory interactions with malaria. The lopinavir carbamate analog resulted in a better inhibitory concentration compared with the lopinavir acetate and lopinavir acetic acid, indicating hydrogen bonding is a crucial interaction.

The biological screening is consistent in measuring the potency of the compound toward the particular malarial strain without disturbance from other variables, such as cell membrane permeability. Each of the analogues should have had the necessary lipophilic character, which is shown in Table 2, to penetrate the cell membrane. With this variable eliminated, the results from the screening are able to focus exclusively on the inhibitory effects for the malarial strains.

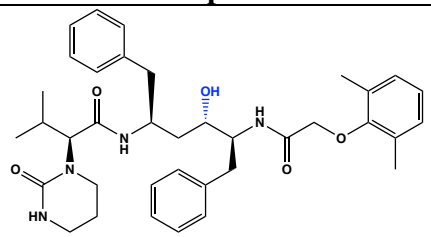
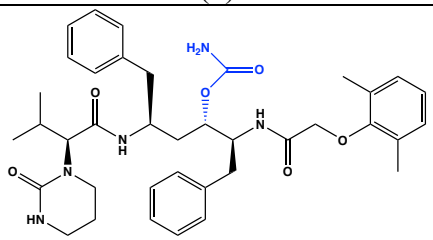
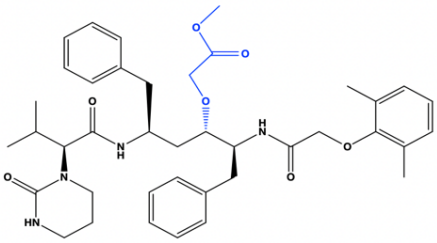
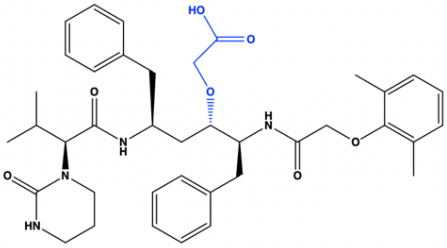
Compound	Molecular Weight	ClogP
 (1)	628.81	6.0946
 (2)	671.84	6.127
 (3)	700.88	6.91
 (4)	686.85	6.0379

Table 2. Chemical properties of lopinavir and synthesized analogs. ClogP estimations from ChemDraw.

Based on the malarial inhibition data, it can be concluded that the secondary hydroxyl group is required for malarial inhibition as modification of this functional group increased the concentration needed for fifty percent inhibition. This indicates the

secondary hydroxy group in the pharmacophore of the protease inhibitor contributes to both the antiretroviral activity and the antimalarial activity. If HIV-1 assay data was available for these compounds, it would most likely show the analogs devoid of HIV-1 inhibitory activity. Additional research is needed to validate the importance of the hydrogen bonding in malarial inhibition. Analogs need to be synthesized with functional groups similar in size to the hydroxyl to explore trends for a lack of hydrogen bonding and for an increase in hydrogen bonding. In this research, only the D6 and W2 strains of malaria were assayed; however, investigation into the most prominent strains in sub-Saharan Africa is needed. Research is also needed to determine the protease inhibitor's mechanism of action for inhibiting the varying strains of *P. falciparum*.

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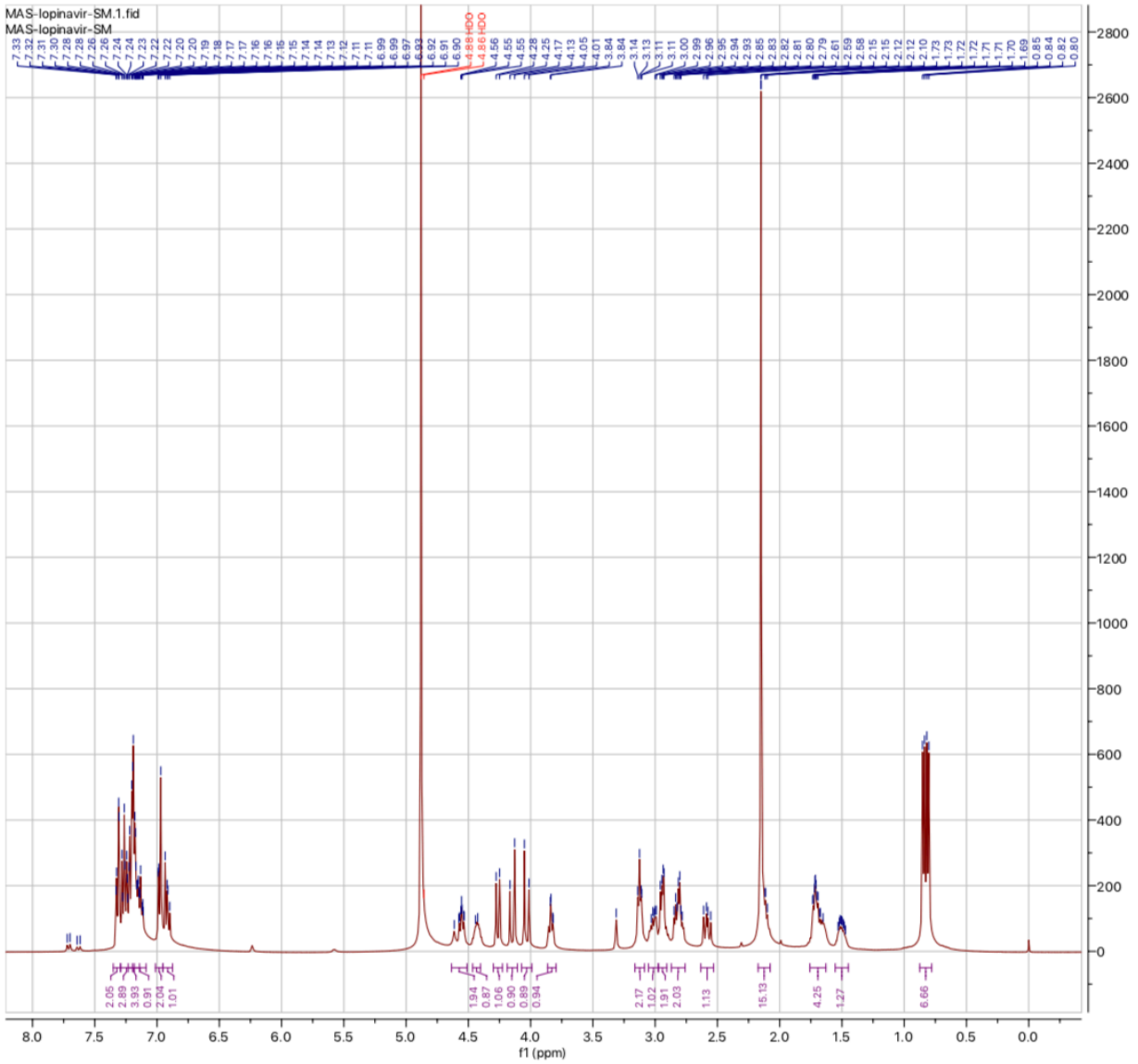
APPENDIX



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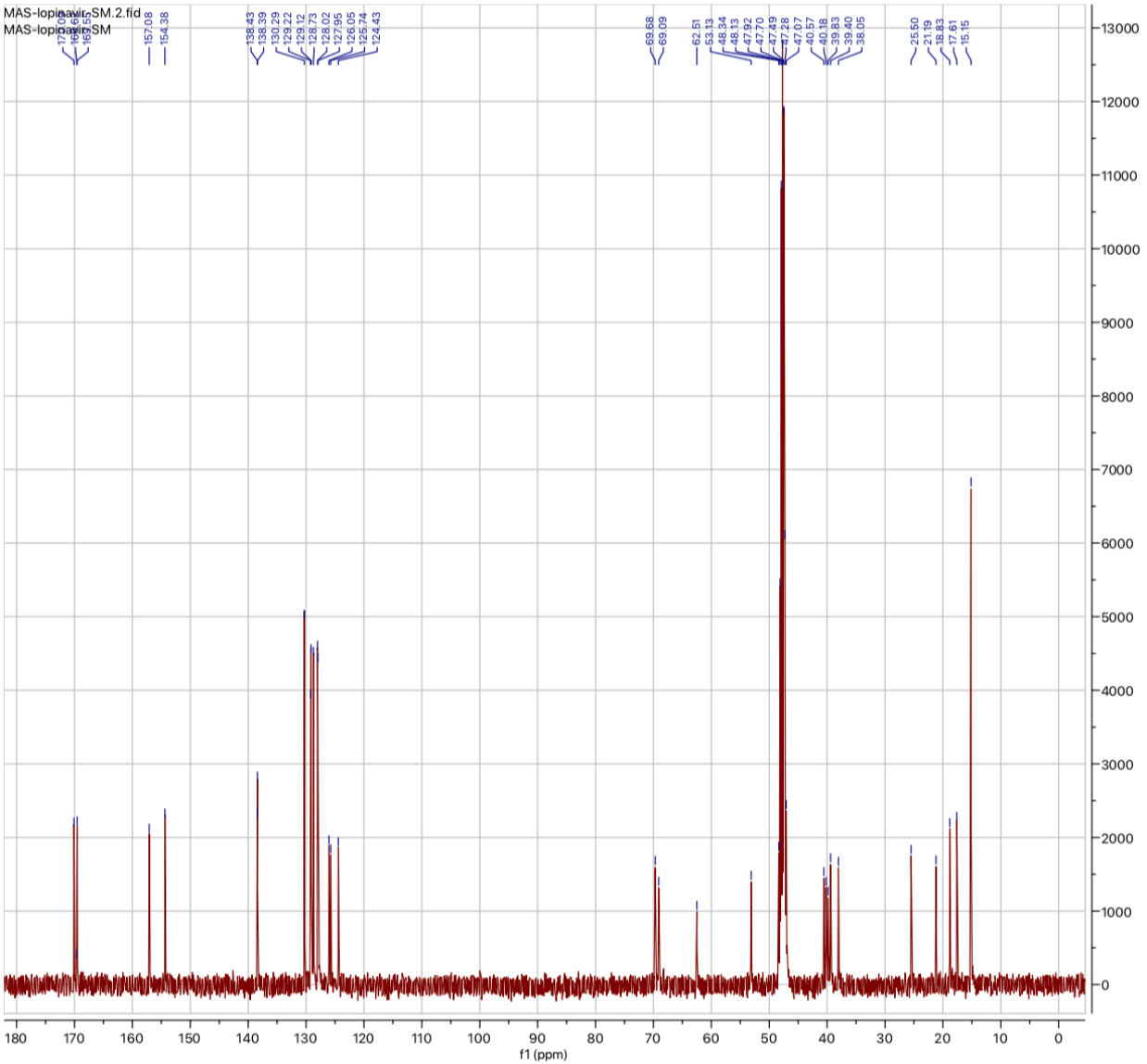
4	Solvent	MeOD
5	Temperature	295.5
6	Pulse Sequence	zg30
7	Experiment	1D
8	Probe	5 mm PABBO BB-1H/ D Z-GRD Z108618/ 0813
9	Number of Scans	64
10	Pulse Width	9.0000
11	Acquisition Time	5.1118
12	Acquisition Date	2018-05-10T12:39:04
13	Spectrometer Frequency	400.17
14	Nucleus	1H

^{1}H NMR (400 MHz, Methanol-d_4) δ 7.35 – 7.29 (m, 2H), 7.29 – 7.20 (m, 3H), 7.19 (ddt, J = 5.9, 3.9, 2.5 Hz, 4H), 7.18 – 7.09 (m, 1H), 6.98 (d, J = 6.6 Hz, 2H), 6.92 (dd, J = 8.6, 6.2 Hz, 1H), 4.64 – 4.51 (m, 2H), 4.43 (d, J = 7.2 Hz, 1H), 4.26 (d, J = 11.1 Hz, 1H), 4.15 (d, J = 14.8 Hz, 1H), 4.03 (d, J = 14.8 Hz, 1H), 3.86 – 3.80 (m, 1H), 3.16 – 3.09 (m, 2H), 3.05 – 2.97 (m, 1H), 2.94 (dd, J = 7.7, 3.8 Hz, 2H), 2.81 (tt, J = 8.1, 4.6 Hz, 2H), 2.58 (dd, J = 13.6, 9.4 Hz, 1H), 2.17 – 2.08 (m, 15H), 1.76 – 1.63 (m, 4H), 1.55 – 1.45 (m, 1H), 0.83 (dd, J = 13.7, 6.6 Hz, 7H).



	Parameter	Value
1	Title	MAS-lopinavir-SM.2.fid
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4	Solvent	MeOD
5	Temperature	296.2
6	Pulse Sequence	zgpg30
7	Experiment	1D
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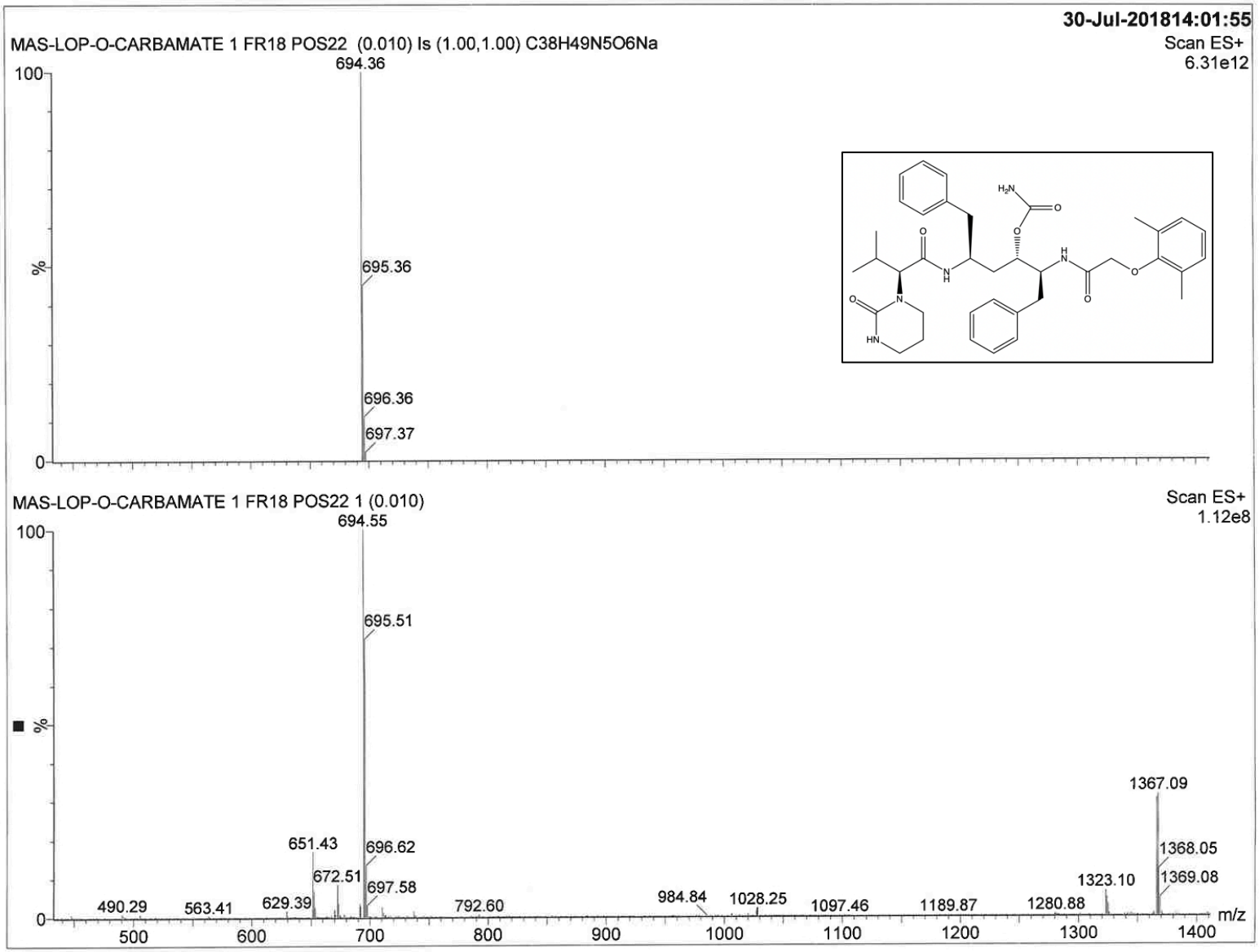
9	Number of Scans	3988
10	Pulse Width	9.0000
11	Acquisition Time	1.3664
12	Acquisition Date	2018-05-10T15:53:24
13	Spectrometer Frequency	100.63
14	Nucleus	13C

^{13}C NMR (101 MHz, MeOD) δ 170.09, 169.62, 169.55, 157.08, 154.38, 138.43, 138.39, 130.29, 129.22, 129.12, 128.73, 128.02, 127.95, 126.05, 125.74, 124.43, 69.68, 69.09, 62.51, 53.13, 48.34, 48.13, 47.92, 47.70, 47.49, 47.28, 47.07, 40.57, 40.18, 39.83, 39.40, 38.05, 25.50, 21.19, 18.83, 17.61, 15.15.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694.36
695.36
696.36
697.37
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1.12e8
694.55
695.51
651.43
696.62
672.51
697.58
490.29
563.41
629.39
792.60
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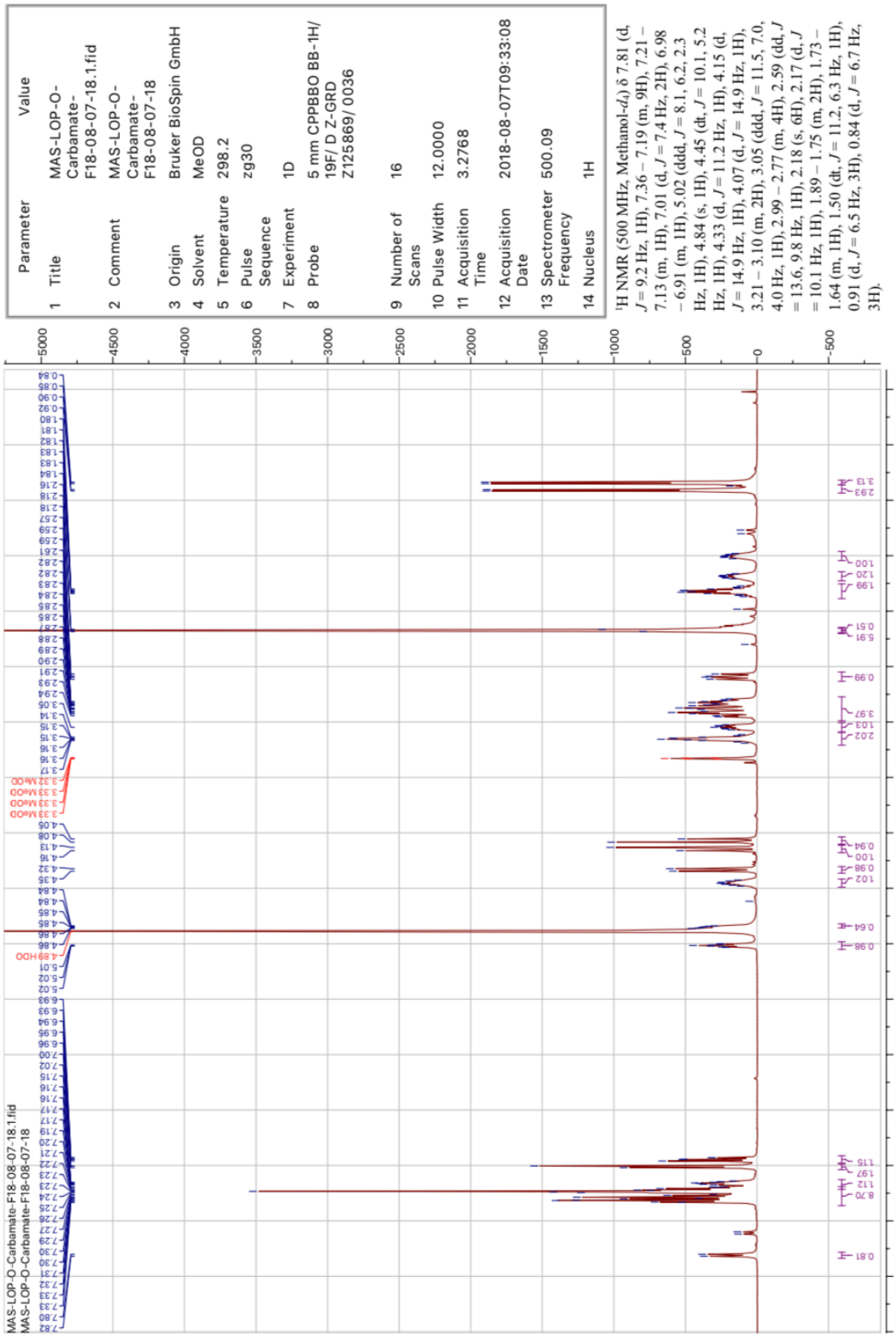
1369.08
m/z



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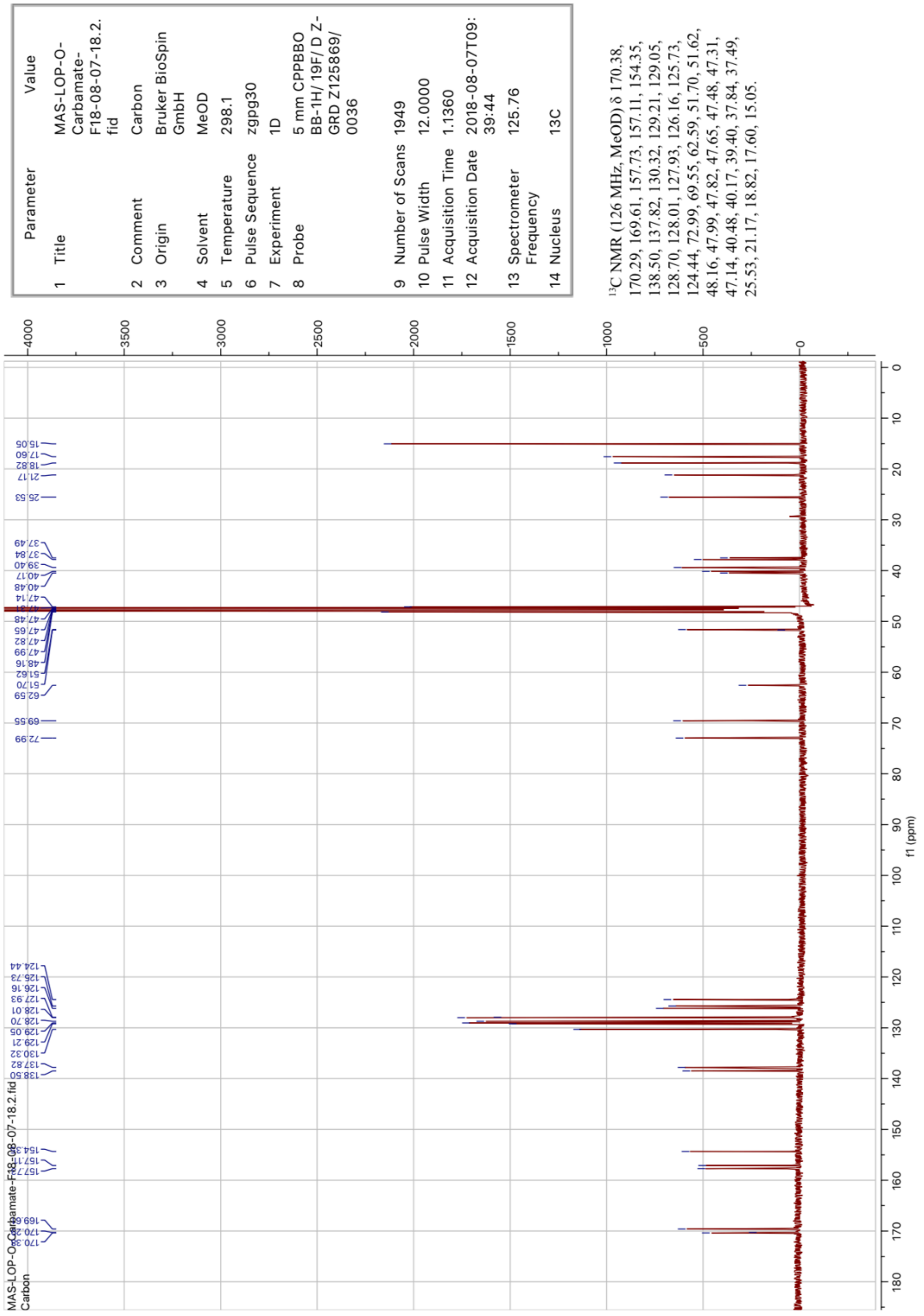
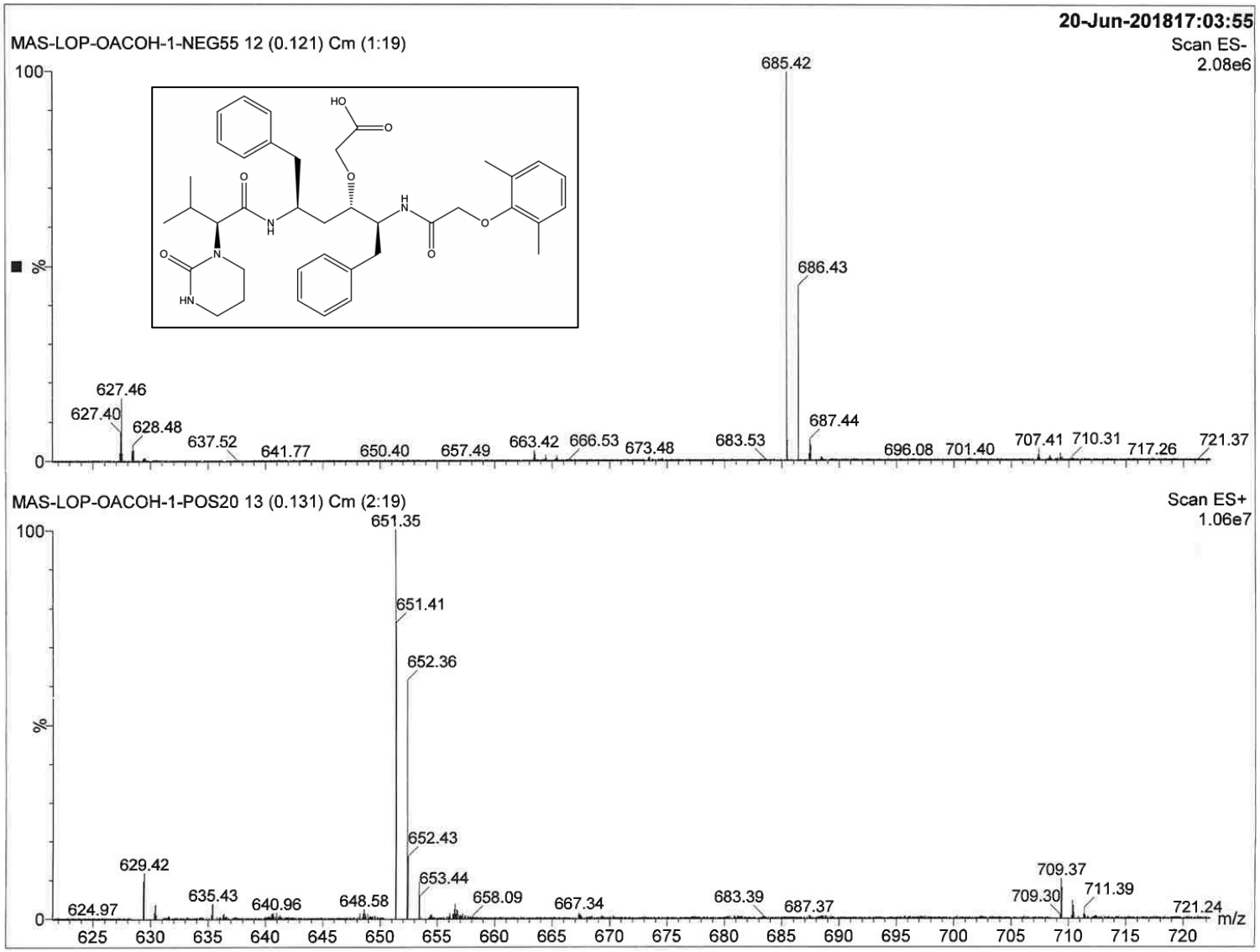
4	Solvent	MeOD
5	Temperature	298.2
6	Pulse Sequence	zg30
7	Experiment	1D
8	Probe	5 mm CPPBBO BB-1H/19F/ D Z-GRD Z125869/ 0036
9	Number of Scans	16
10	Pulse Width	12.0000
11	Acquisition Time	3.2768
12	Acquisition Date	2018-08-07T09:33:08
13	Spectrometer Frequency	500.09
14	Nucleus	1H

^{1}H NMR (500 MHz, Methanol-d_4) δ 7.81 (d, J = 9.2 Hz, 1H), 7.36 – 7.19 (m, 9H), 7.21 – 7.13 (m, 1H), 7.01 (d, J = 7.4 Hz, 2H), 6.98 – 6.91 (m, 1H), 5.02 (ddd, J = 8.1, 6.2, 2.3 Hz, 1H), 4.84 (s, 1H), 4.45 (dt, J = 10.1, 5.2 Hz, 1H), 4.33 (d, J = 11.2 Hz, 1H), 4.15 (d, J = 14.9 Hz, 1H), 4.07 (d, J = 14.9 Hz, 1H), 3.21 – 3.10 (m, 2H), 3.05 (ddd, J = 11.5, 7.0, 4.0 Hz, 1H), 2.99 – 2.77 (m, 4H), 2.59 (dd, J = 13.6, 9.8 Hz, 1H), 2.18 (s, 6H), 2.17 (d, J = 10.1 Hz, 1H), 1.89 – 1.75 (m, 2H), 1.73 – 1.64 (m, 1H), 1.50 (dt, J = 11.2, 6.3 Hz, 1H), 0.91 (d, J = 6.5 Hz, 3H), 0.84 (d, J = 6.7 Hz, 3H).



^{13}C NMR (126 MHz, MeOD) δ 170.38, 170.29, 169.61, 157.73, 157.11, 154.35, 138.50, 137.82, 130.32, 129.21, 129.05, 128.70, 128.01, 127.93, 126.16, 125.73, 124.44, 72.99, 69.55, 62.59, 51.70, 51.62, 48.16, 47.99, 47.82, 47.65, 47.48, 47.31, 47.14, 40.48, 40.17, 39.40, 37.84, 37.49, 25.53, 21.17, 18.82, 17.60, 15.05.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627.40
628.48
637.52
641.77
650.40
657.49
663.42
666.53
673.48
683.53
687.44
696.08
701.40
707.41
710.31
717.26
721.37
MAS-LOP-OACOH-1-POS20 13 (0.131) Cm (2:19)
Scan ES+
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100
%
0
651.35
651.41
652.36
652.43
629.42
653.44
709.37
709.30
711.39
624.97
635.43
640.96
648.58
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667.34
683.39
687.37
721.24
m/z
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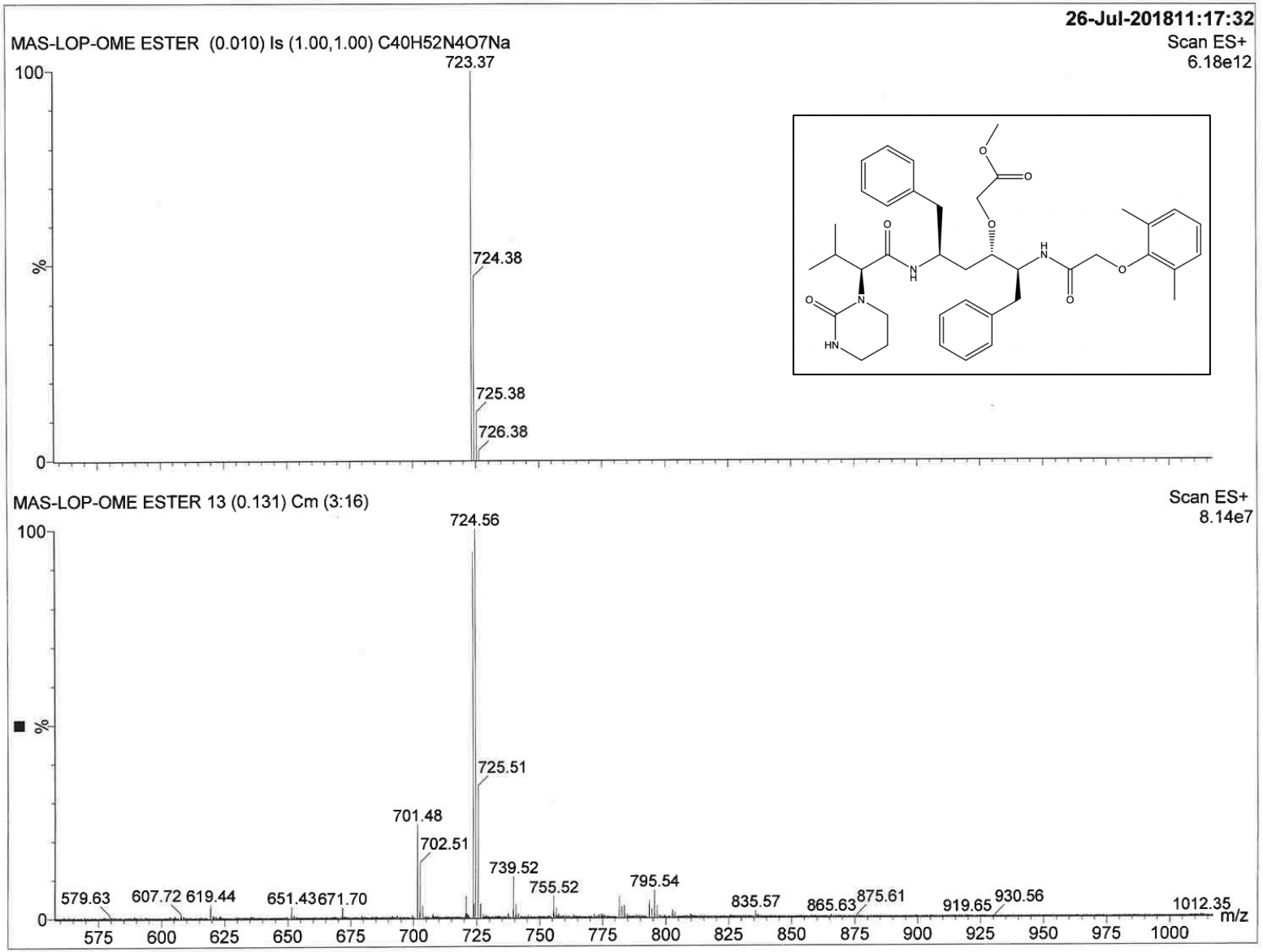
705
710
715
720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%
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725.38
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725.51
701.48
702.51
739.52
755.52
795.54
579.63
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925
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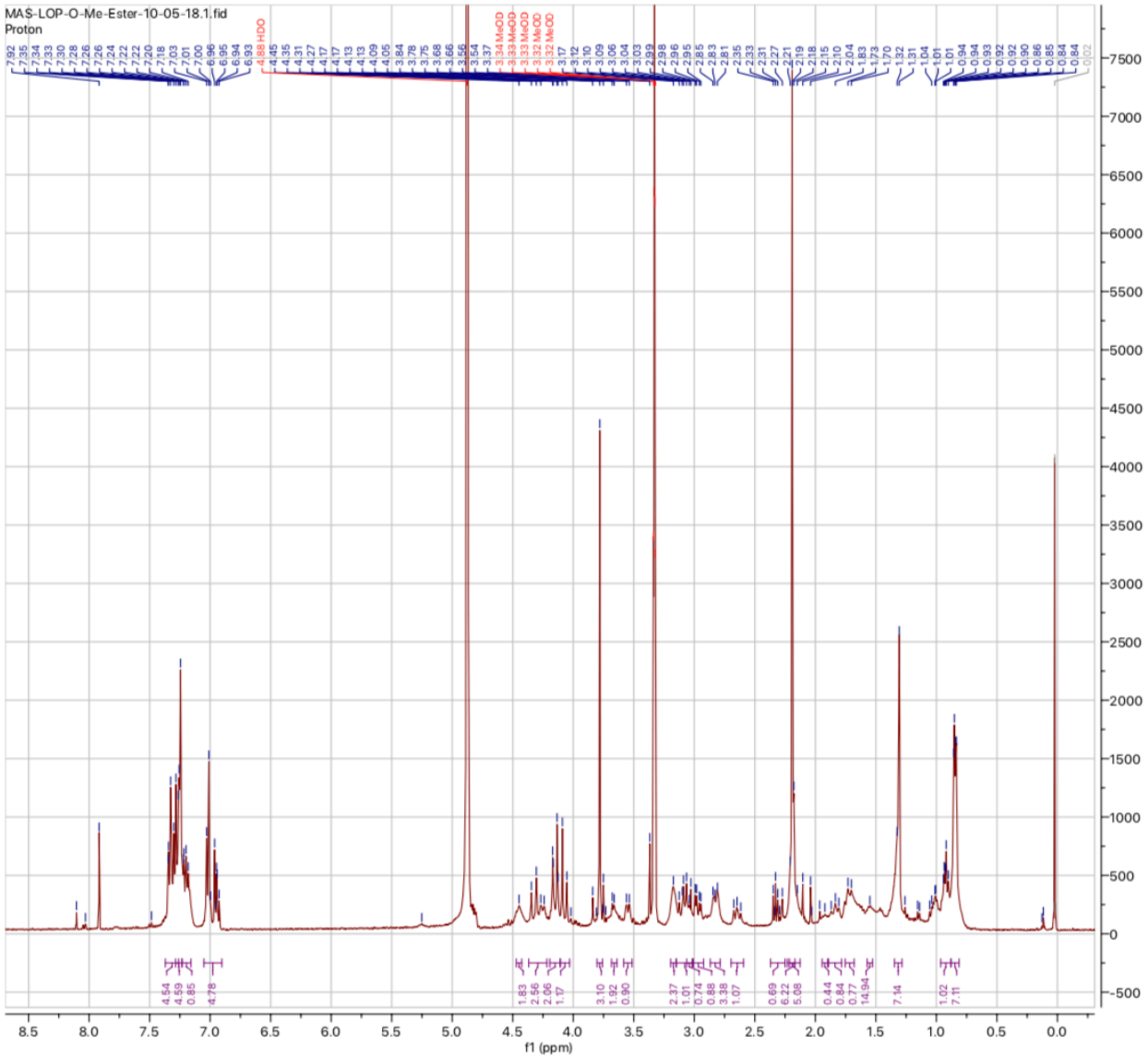

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6	Pulse Sequence	zg30
7	Experiment	1D
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9	Number of Scans	84
10	Pulse Width	11.2500
11	Acquisition Time	4.0894
12	Acquisition Date	2018-10-05T12:33:58
13	Spectrometer Frequency	400.18
14	Nucleus	1H

^{1}H NMR (400 MHz, Methanol-d_4) δ 7.31 (dd, J = 17.5, 7.2 Hz, 5H), 7.25 (d, J = 4.4 Hz, 5H), 7.23 – 7.16 (m, 1H), 7.05 – 6.90 (m, 5H), 4.45 (s, 2H), 4.29 (dd, J = 28.5, 13.7 Hz, 3H), 4.19 – 4.11 (m, 2H), 4.07 (d, J = 14.8 Hz, 1H), 3.78 (s, 3H), 3.66 (s, 2H), 3.55 (d, J = 9.5 Hz, 1H), 3.17 (s, 2H), 3.15 – 3.01 (m, 1H), 3.04 (s, 1H), 2.97 (dd, J = 13.9, 4.9 Hz, 1H), 2.87 – 2.79 (m, 3H), 2.70 – 2.59 (m, 1H), 2.37 – 2.25 (m, 1H), 2.19 (d, J = 11.9 Hz, 5H), 2.19 (s, 6H), 1.92 (s, 0H), 1.84 (t, J = 12.6 Hz, 1H), 1.72 (d, J = 11.3 Hz, 1H), 1.55 (s, 15H), 1.32 (d, J = 6.8 Hz, 7H), 0.96 – 0.88 (m, 1H), 0.85 (dd, J = 6.7, 2.5 Hz, 7H).